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14. ABSTRACT

We are investigating whether inflammation can enhance prostate carcinogenesis in a rat model of dietary charred meat carcinogen induced cancers, and, whether antioxidant and other chemopreventative compounds can reduce prostate cancer in this model. In this period we have extended our rat studies to show that PhIP will cause prostatic intraepithelial neoplasia in the mouse, providing a greatly enhanced ability to study molecular mechanisms of PhIP induced prostate carcinogenesis and for prevention of prostate cancer induced by PhIP. We have also began to extensively characterize another mouse model of prostate cancer that we will also be able to used in studies of PhIP induced prostate cancer and prevention. Finally, we have begun to extend our studies to understanding the molecular mechanisms of PhIP induced prostate cancer by developing an assay to detect CpG dinucleotide hypermethyalation in the rat GSTP1 promoter. These studies set the stage for us to complete all of our stated aims in year 3 of this proposal.

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Prostate cancer, PhIP, chemoprevention

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INTRODUCTION

Our overall goal is to determine whether prostate cancer is driven by a combination of inflammation and dietary carcinogens and to develop methods to interrupt the disease process using preventative measures. Several studies have demonstrated that the consumption of specific heterocyclic amine compounds that are produced from the charring or over-cooking of meats, like 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), is associated with prostate cancer. Interestingly, new evidence from the fields of population and genetic epidemiology, molecular pathology and molecular genetics has brought attention to the possible role of inflammation in prostate carcinogenesis. Utilizing a novel method of inciting rodent prostate inflammation developed by our lab, in an established animal model of PhIP induced prostate cancer, we found that viral induced prostate inflammation can modulate the frequency with which prostate DNA mutations occur and, when incited in conjunction with dietary exposure to PhIP, can further increase prostate DNA mutation frequency and lead to chronic prostate inflammation. In this award we will determine if inflammation can augment carcinogenesis is this rat model and if certain dietary chemo preventative agents can prevent cancer in this model.

BODY

The aims of this proposal were to:

Aim (1) Evaluation of candidate dietary prostate cancer chemopreventive agents (broccoli tea, soy protein, vitamin E, lycopene) for their ability to alter DNA mutagenesis and chronic prostate inflammation in a novel animal model of chronic prostate inflammation.

Aim (2) Determination of the capacity of alternative methods of inciting prostate inflammation to induce chronic inflammation in the context of dietary consumption of PhIP.

Aim (3) Determination of the ability of viral induced prostate inflammation to accelerate prostate carcinogenesis in a rat model of dietary (PhIP) induced prostate cancer.

In order to accomplish these aims, we outlined the following tasks:

Task 1 *Assessment of the ability of candidate dietary prostate cancer chemopreventive compounds (e.g., broccoli tea, soy, vitamin E, lycopene) to alter DNA mutagenesis and chronic prostate inflammation in an animal model, Months 1-12:*

The main aspects of this task will be completed in year 3 of this award. In relation to this task we have begun and generated substantial progress in a new but related avenue of research designed to extend our studies of dietary prevention of PhIP induced prostate carcinogenesis from the rat only into the mouse. There are numerous reasons for this

including the fact that mice are much more amendable to genetic manipulation which will allow us ultimately to study the molecular pathways that are altered in response to PhIP that leads to PIN and prostate cancer development.

Further, since PhIP induced prostate cancers remain as early lesions that do not metastasize we reasoned that adding PhIP to the Lo-MYC model of early prostate cancer (Cancer Cell. 4:223-238, 2003), which also does not metastasize, might induce more aggressive prostate cancers that do metastasize. This would, therefore, better recapitulate more of the natural history of prostate cancer in humans.

As a first approach and prerequisite to develop this model we have been characterizing the Lo-MYC mice originally developed by Charles Sawyers et al. This work, outlined below, has been submitted as an abstract to the Annual Meeting of the American Urological Association (see appendix).

Introduction and Objective: *C-MYC* has been implicated in human prostate cancer and targeted overexpression of *C-MYC* in the mouse results in prostatic intraepithelial neoplasia (PIN) and invasive adenocarcinoma (CaP) (Cancer Cell. 4:223-238, 2003). However, the onset and dynamics of C-MYC protein expression, and its relation to morphological transformation, the expression of other prostate tumor suppressor proteins, early invasion and the development of inflammation have not been addressed.

Methods: Lo-MYC and wild type FVB mice were obtained from the NCI Mouse Models of Human Cancer Repository and fed normal chow. Mice were sacrificed at various ages up to 52 weeks. The prostate lobes were dissected and processed for histological and immunohistochemical analysis for C-MYC, androgen receptor (AR), Nkx 3.1, p27, Ki67, cleaved caspase 3, F4/80, CD3, CD45, p63 and smooth muscle actin.

Results: All mice showed PIN at 4 weeks of age with no invasion until 52 weeks. The morphological alterations in PIN included an increase in cell and nuclear size, nucleolar enlargement, hyperchromasia, increased mitoses and apoptotic bodies. Detection of C-MYC protein coincided with morphological alterations, suggesting that C-MYC is sufficient for transformation. All mice showed microinvasive CaP by 52 weeks, and both the level of C-MYC protein and the degree of cytological atypia were decreased upon early invasion, with recovery of C-MYC protein and nuclear atypia upon enlargement of the invasive tumor. Gland formation and AR expression were maintained during early invasion. The expression of Nkx 3.1 was inverse to that of C-MYC (See Fig. 1 for C-MYC staining). Decreased p27 and increased Ki67 and cleaved caspase 3 were found in PIN and CaP. Inflammatory cells, consisting of mostly of CD45 positive lymphocytes and F4/80 positive macrophages increased in an around the lesions as they progressed.

Conclusions: We verified that Lo-MYC mice develop PIN and early CaP that resemble the human disease. Our new data show: (i) C-MYC is sufficient to morphologically transform prostate cells into PIN; (ii) C-MYC protein is decreased and Nkx3.1 protein is increased transiently during invasion; (iii) inflammatory cell infiltrates accompany the development and progression of PIN to CaP. These results validate the ability of the

Lo-MYC mouse to model early human CaP and reveal dynamics of C-MYC and Nkx3.1 protein expression and inflammation during disease progression. We propose below to use these mice to determine whether addition of PhIP will accelerate the disease further, possibly by enhancing inflammation, and then to test our chemopreventative strategies on these mice.

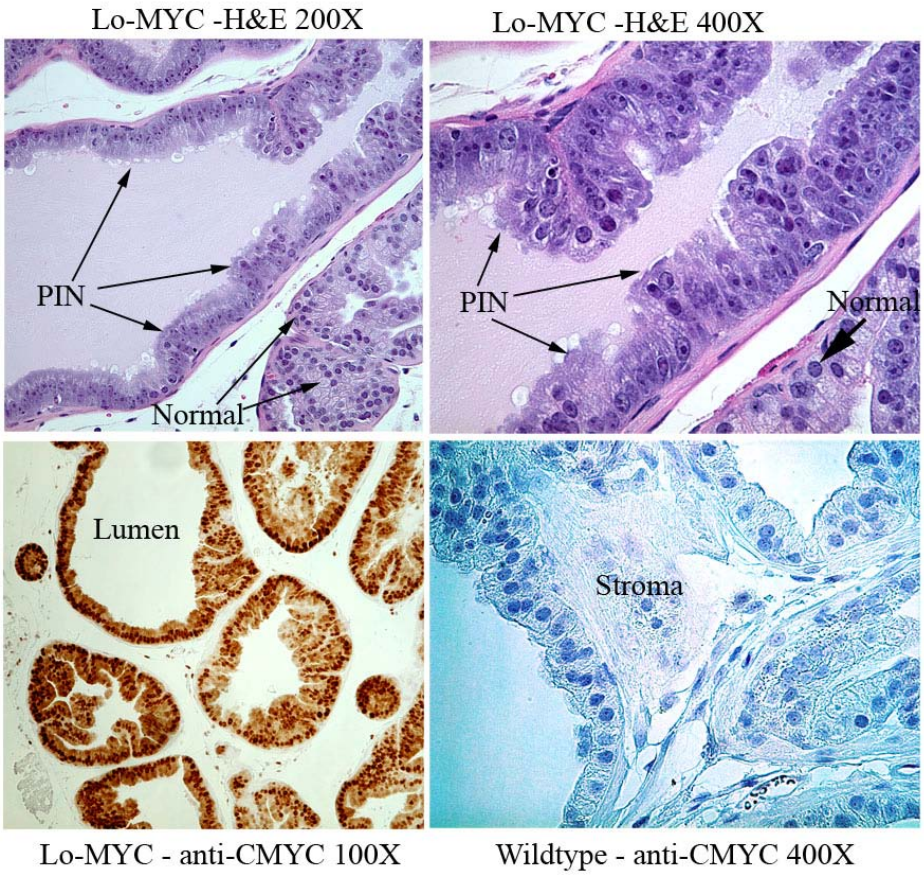

Figure 1. Histology in 8 Week Lo-MYC Mice. Note markedly enlarged nucleoli in hematoxylin and eosin stained sections (H&E) in Lo-MYC mouse. Note strong nuclear C-MYC staining in the epithelial cells in the Lo--MYC mice. Wildtype mice show no C-MYC staining, even at higher power.

Task 2: Determination of the effect of alternative methods of inducing prostate inflammation on the development of chronic inflammation in an established animal model, Months 12-19.

We have not begun task 2 as yet. We are planning on completing this in year 3.

Task 3: Determination of the ability of viral induced prostate inflammation to accelerate prostate carcinogenesis in a rat model of dietary (PhIP) induced prostate cancer, Months 12-36.

We are still in the process of evaluating whether the treatments affected the frequency and extent of PIN and intraductal carcinoma in the ventral lobes and as secondary objectives we will determine the frequency of other cancers, such as colo-rectal cancer and lymphoma. We have obtained an Aperio Slide scanning system that will facilitate the studies of quantifying the extent of the various lesions: PIN, intraductal carcinoma and invasive carcinoma. The results of this study will serve as a baseline for the remaining studies in task 3 in terms of the number and pattern of prostate lesions expected at 52 weeks after 20 weeks of PhIP treatment in our hands and a guide for all other studies outlined in the proposal.

In a related work we are also interested in developing an understanding of the molecular alterations that occur in PhIP induced prostate neoplastic lesions to determine whether the same somatic genomic alterations that occur in human prostate cancer also occur in this rat model. Toward this end during this past year of funding we have optimized PCR primers to perform bisulfite DNA sequencing of a region of the CpG island of the rat *GSTP1* promoter and have begun to test whether this bisulfite sequencing reaction will be successful after extracting DNA from paraffin blocks from rat prostate tissues. Our plan is to perform laser capture microdissection on the PIN and early carcinoma lesions and compare the results of this to both normal epithelium in treated and untreated rats, as well as to potential changes seen in atrophy that has been induced by PhIP. This information will be used for our prevention aims to monitor molecular changes in PhIP induced cancers as well as morphological changes as originally outlined.

KEY RESEARCH ACCOMPLISHMENTS

- Obtained and extensively characterized, both morphologically and immunohistochemically, the Lo-MYC model of human prostate cancer in order to ultimately use this model in studies of dietary prevention of PhIP induced cancers.

- Developed a bisulfite DNA sequencing strategy to determine whether *GSTP1* promoter hypermethylation accompanies prostate carcinogenesis in the rat as it does in the human.

- We published a major review article on prostate cancer and inflammation in *Nature Reviews Cancer*, which outlined some of our DOD sponsored research. See Appendix.

- Other accomplishments of the research team related to this funded project.

    - Our team continues to work and collaborate effectively on studies to examine the etiology of prostate cancer and other prostate diseases. For example, in our main collaborator's laboratory, that of William G. Nelson MD PhD, preliminary results as examined by Dr. De Marzo show that mice that were fed PhIP for 20 weeks indeed develop high grade PIN lesions in the ventral prostate after 52 weeks total. This work lays the ground work for our future mouse studies. In addition, Dr. Nelson's laboratory has also obtained preliminary data showing that treatment of neonatal transgenic Lo-MYC mice with the synthetic estrogen, Diethylstibestrol (DES), results in the development of increased levels of inflammation in the mouse ventral prostate as compared to levels induced by DES in wild-type mice or in induced by C-MYC overexpression alone. Thus, we have available another potential model of inducing inflammation in the rodent prostate.

- Shown below are publications since the submission of the last progress report.

Nelson WG, Yegnasubramanian S, Agoston AT, Bastian PJ, Lee BH, Nakayama M, De Marzo AM. Abnormal DNA methylation, epigenetics, and prostate cancer. Front Biosci. 12: 4254-66. 2007.

De Marzo AM, Nakai Y, Nelson WG. Inflammation, atrophy, and prostate carcinogenesis. Urol Oncol. 25:398-400, 2007.

Grosso JF, Kelleher CC, Harris TJ, Maris CH, Hipkiss EL, De Marzo A, Anders R, Netto G, Getnet D, Bruno TC, Goldberg MV, Pardoll DM, Drake CG. LAG-3 regulates CD8 T cell accumulation and effector function in murine self- and tumor-tolerance systems. J Clin Invest. 2007.

Sutcliffe S, Rohrmann S, Giovannucci E, Nelson KE, De Marzo AM, Isaacs WB, Nelson WG, Platz EA. Viral infections and lower urinary tract symptoms in the third national health and nutrition examination survey. J Urol. 178:2181-5, 2007.

Sutcliffe S, Giovannucci E, Gaydos CA, Viscidi RP, Jenkins FJ, Zenilman JM, Jacobson LP, De Marzo AM, Willett WC, Platz EA. Plasma antibodies against Chlamydia trachomatis, human papillomavirus, and human herpesvirus type 8 in relation to prostate cancer: a prospective study. Cancer Epidemiol Biomarkers Prev. 16:1573-80, 2007.

Kachhap SK, Faith D, Qian DZ, Shabbeer S, Galloway NL, Pili R, Denmeade SR, DeMarzo AM, Carducci MA. The N-Myc down regulated Gene1 (NDRG1) Is a Rab4a effector involved in vesicular recycling of E-cadherin. PLoS ONE. 2:e844, 2007.

REPORTABLE OUTCOMES

- Completed manuscript:

De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg H, Drake CG, Nakai Y, Isaacs WB, and Nelson WG. Inflammation in prostate carcinogenesis. Nat Rev Can 7(4):256-269, 2007.

- Abstract:

Tsuyoshi Iwata, Charles J. Bieberich, Angelo M De Marzo. Paradoxical downregulation of C-MYC and upregulation of NKX3.1 proteins during invasion in the Lo-MYC mouse prostate. Submitted to the American Urological Association Annual Meeting, Orlando FL, May 16-22, 2008.

CONCLUSIONS

The results of studies carried out so far reveal that we are poised to extend our rodent studies of PhIP induced prostate cancer and dietary prevention of such into mouse models. We have also developed technology to monitor key molecular alterations that accompany prostate carcinogenesis in these models. This will facilitate an expanded understanding of the molecular pathogenesis of PhIP induced prostate cancer and our ability to prevent it. These studies will guide and extend our ability to complete our remaining studies as outlined in the original proposal.

REFERENCES

- None

APPENDICES

- Completed manuscript:

De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg H, Drake CG, Nakai Y, Isaacs WB, and Nelson WG. Inflammation in prostate carcinogenesis. Nat Rev Can 7(4):256-269, 2007.

Inflammation in prostate carcinogenesis

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Abstract | About 20% of all human cancers are caused by chronic infection or chronic inflammatory states. Recently, a new hypothesis has been proposed for prostate carcinogenesis. It proposes that exposure to environmental factors such as infectious agents and dietary carcinogens, and hormonal imbalances lead to injury of the prostate and to the development of chronic inflammation and regenerative 'risk factor' lesions, referred to as proliferative inflammatory atrophy (PIA). By developing new experimental animal models coupled with classical epidemiological studies, genetic epidemiological studies and molecular pathological approaches, we should be able to determine whether prostate cancer is driven by inflammation, and if so, to develop new strategies to prevent the disease.

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Prostate cancer is the most common non-cutaneous malignant neoplasm in men in Western countries, responsible for the deaths of approximately 30,000 men per year in the United States[1]. The number of afflicted men is increasing rapidly as the population of males over the age of 50 grows worldwide. Therefore, finding strategies for the prevention of prostate cancer is a crucial medical challenge. As men in South East Asian countries have a low incidence of prostate cancer that increases rapidly after immigration to the West, this disease is not an intrinsic feature of ageing. The pathogenesis of prostate cancer reflects both hereditary and environmental components. What are the environmental factors and genetic variations that have produced such an epidemic of prostate cancer? Approximately 20% of all human cancers in adults result from chronic inflammatory states and/or chronic inflammation[2–4] (BOX 1), which are triggered by infectious agents or exposure to other environmental factors, or by a combination thereof. There is also emerging evidence that inflammation is crucial for the aetiology of prostate cancer. This evidence stems from epidemiological, histopathological and molecular pathological studies. The objective of this Review is to take a multidisciplinary approach to present and analyse such studies. Because several reviews related to these topics have been published[5–7], here we will focus on new findings and ideas with the purpose of sparking innovative areas of investigation that might ultimately lead to the prevention of prostate cancer.

Enigmas in the aetiology of prostate cancer

As in other cancers, prostate cancer develops through the accumulation of somatic genetic and epigenetic changes, resulting in the inactivation of tumour-suppressor genes and caretaker genes, and the activation of oncogenes[8,9] (TABLE 1). There is also evidence for an underlying genetic instability that might facilitate tumour progression[10,11]. Although these genetic and epigenetic changes are crucial for our understanding of 'how' prostate cancer arises, another key remaining question is 'why' prostate cancer is so common. The most consistent risk factors for the development of prostate cancer are advancing age, family history and race — diet is thought to be an emerging risk factor. To answer the question of why prostate cancer is so prevalent, several puzzling facts regarding its occurrence must be explained. The first enigma is the striking organ selectivity of prostate cancer within the genitourinary system: whereas there are approximately 280,000 new cases of prostate cancer in the US each year, there have been less than 50 reported cases of primary seminal vesicle carcinoma in the English literature[12]. The second unexplained issue is the geographic variation in the incidence of prostate cancer: as compared with the US and Western Europe, the incidence and mortality rates for prostate cancer are much lower in Southeast and East Asia[13]. Chinese and Japanese men who immigrate to the west acquire higher prostate cancer risks within one generation[14], supporting an effect of the environment

At a glance

- Prostate cancer is the most common form of non-skin cancer in men in developed countries. The cause(s) of prostate cancer have not yet been clarified. Although heritable factors are implicated, immigration studies indicate that environmental exposures are also important.
- Chronic infection and inflammation cause cancer in several organs including the stomach, liver and large intestine. Data from histopathological, molecular histopathological, epidemiological and genetic epidemiological studies show that chronic inflammation might also be important in prostate carcinogenesis.
- The source of intraprostatic inflammation is often unknown, but might be caused by infection (for example, with sexually transmitted agents), cell injury (owing to exposure to chemical and physical trauma from urine reflux and prostatic calculi formation), hormonal variations and/or exposures, or dietary factors such as charred meats. The resultant epithelial cellular injury might cause a loss of tolerance to normal prostatic antigens, resulting in a self-perpetuating autoimmune reaction.
- Exposures to infectious agents and dietary carcinogens are postulated to directly injure the prostate epithelium, resulting in the histological lesions known as proliferative inflammatory atrophy (PIA), or proliferative atrophy. These lesions are postulated to be a manifestation of the 'field effect' caused by environmental exposures.
- Despite a strong genetic component to prostate cancer risk, no highly penetrant hereditary prostate cancer genes have been uncovered to date. Although complex, genetic variation in inflammatory genes is associated with prostate cancer risk.
- Several challenges remain regarding the inflammation hypothesis in prostate cancer, including the determination of the cause(s) of chronic inflammation in the prostate, an understanding of the cellular and molecular biology of the immune response in the prostate, whether inflammatory cells are truly causative in the process, and the determination of the target cell types within the proposed precursor lesions of prostate cancer.
- The refinement and application of new epidemiological approaches, including high-throughput genetic epidemiology, improved rodent models of prostate inflammation and cancer, and advances in the application of molecular techniques to histopathological studies should provide insights into the cause of prostate inflammation and its relevance to prostate carcinogenesis.

on prostate cancer development. A third key unsolved problem is the zonal predilection of prostate cancer. Most cancer lesions occur in the peripheral zone of the gland, fewer occur in the transition zone, and almost none arise in the central zone[15] (FIG. 1).

Inflammation and prostate cancer: the role of PIA

Histologically, most lesions that contain either acute or chronic inflammatory infiltrates in the prostate are associated with atrophic epithelium or focal epithelial atrophy[16–18]. Perhaps correspondingly, focal areas of epithelial atrophy are common in the ageing prostate[16,19], and often encompass a large fraction of the peripheral zone, where atrophy most often occurs[19,20]. Compared with normal epithelium, there is an increased fraction of epithelial cells that proliferate in focal atrophy lesions[18,21,22], and we have proposed the term proliferative inflammatory atrophy (PIA) for most of these atrophic lesions[18,23]. Not all focal prostate atrophy lesions show increased inflammatory cells, and for these the term proliferative atrophy might be used. In morphological studies we and others have observed transitions between atrophic epithelium and adenocarcinoma[16,24,25], and frequent transitions between areas of PIA and/or proliferative atrophy with high grade prostatic intraepithelial neoplasia (PIN)[18,26]. Although there is evidence for somatic genetic changes in PIA and proliferative atrophy, it seems from the studies published so far that most PIA and proliferative atrophy lesions do not harbour clonal genetic alterations (BOX 2). Tissue samples from patients with benign prostate hyperplasia (BPH), which occurs in the transition zone of the prostate (FIG. 1), have areas with markedly increased numbers of chronic inflammatory cells. In these areas, in almost all cases, the epithelium seems to be atrophic, indicating that these regions can be considered PIA of the transition zone.

Several key molecular pathways involved in prostate cancer have also been shown to be altered in PIA lesions (BOX 3). For example, the protein products of three prostate tumour-suppressor genes: *NKX3.1* (REF. 27), *CDKN1B*, which encodes p27 (REFS 18,23), and phosphatase and tensin homologue (*PTEN*) (A.M.D. and D. Faith, unpublished observations) are all downregulated in focal atrophy lesions. These genes are highly expressed in normal prostate epithelium, and frequently decreased or absent in PIN and prostate cancer. In addition, one allele of their corresponding genetic loci is frequently deleted in carcinomas (TABLE 1), and forced overexpression of each of these genes causes decreased growth of prostate cancer cells in culture. Finally, animal models with targeted disruption of either one or two alleles of the corresponding mouse genes develop prostate hyperplasia, PIN and/or invasive carcinoma[28].

What is the source of prostatic inflammation?

In most cases, the cause of prostatic inflammation is unclear. Various potential sources exist for the initial inciting event, including direct infection, urine reflux inducing chemical and physical trauma, dietary factors, oestrogens, or a combination of two or more of these factors (FIG. 2). Furthermore, any of these could lead to a break in immune tolerance and the development of an autoimmune reaction to the prostate.

Infectious agents. Many different pathogenic organisms have been observed to infect and induce an inflammatory response in the prostate. These include sexually transmitted organisms, such as *Neisseria gonorrhoeae*[29], *Chlamydia trachomatis*[30], *Trichomonas vaginalis*[31] and *Treponema pallidum*[32], and non-sexually transmitted bacteria such as *Propionibacterium acnes*[33] and those known to cause acute and chronic bacterial prostatitis, primarily Gram-negative organisms such as *Escherichia coli*[34]. Although each of these pathogens has been identified in the prostate, the extent to which they typically infect this organ varies. For example, *T. pallidum* is a very rare cause of granulomatous prostatitis[32], which is itself a rare pattern of prostate inflammation. In the pre-antibiotic era before 1937, a large proportion of other sexually transmitted infections (STIs, predominantly gonorrhea) resulted in severe prostatic inflammation or prostatic abscess[29]. However, since the introduction of antibiotics this proportion has decreased dramatically, presumably owing to treatment before progression to the prostate. Despite this decline, asymptomatic infection and inflammation of the prostate can still occur. In their study of gonorrhea,

Prostatic intraepithelial neoplasia
A lesion characterized by cells with neoplastic features, which line pre-existing acini and ducts. PIN represents the most likely precursor to many prostate cancers.

Benign prostatic hyperplasia
Non-cancerous enlargement consisting of excess glands and stroma affecting the transition zone of the prostate.

Urine reflux
During urination, urine flows from the bladder through the prostatic urethra and into the penile urethra. Urine reflux occurs when urine flows inadvertently into the prostatic ducts, permeating large portions of the prostatic acini.

Box 1 | **Molecular mechanisms of inflammation-induced cancers**

Chronic inflammation is implicated in the development of a diverse range of human cancers, with overwhelming evidence causally linking it to cancer of the liver, stomach, large intestine, biliary tree and urinary bladder, and significant evidence to link it to cancer of the oesophagus, lung and pancreas. Many of these cancers are associated with infectious agents and/or a defined environmental exposure(s). Inflammation often collaborates with environmental exposures, such as dietary derived toxins, to increase cancer risk even further[132]. The molecular mechanisms that underlie the pathogenesis of inflammation-associated cancer are complex, and involve both the innate and adaptive immune systems[3,133,134,135]. Although viral oncogenes can contribute directly to neoplastic transformation, neither infection nor pathogen-encoded oncogenes are required for inflammatory cells to induce cancer[136]. Indeed, highly reactive chemical compounds, including superoxide, hydrogen peroxide, singlet oxygen and nitric oxide are released from activated phagocytic inflammatory cells of the innate immune system, and can cause oxidative or nitrosative damage to DNA in the epithelial cells, or react with other cellular components such as phospholipids, initiating a free-radical chain reaction[2]. The result is that many host epithelial cells are damaged and killed, and in order to preserve the barrier function of epithelia, these cells must be replaced by cell division from resident progenitor and/or stem cells. Epithelial cells that undergo DNA synthesis in the setting of these DNA-damaging agents are at an increased risk of mutation. That oxidant or nitrosative stress is important for driving prostate cancer formation is bolstered by epidemiological data, which indicate that the consumption of certain types of dietary antioxidants is associated with reduced prostate cancer risk[137]. Inflammatory cells also secrete cytokines that promote epithelial cell proliferation and stimulate angiogenesis[138]. In terms of disease progression, inflammatory cells migrate readily through the extracellular matrix as a result of the release of proteolytic enzymes and their inherent motile nature. Therefore, they might facilitate epithelial cell invasion into the stromal and vasculature compartments and, ultimately, the metastasis of tumour cells[133,134]. In another mechanism, the disruption of cytokine production and regulation, including cytokine deficiencies, lead to increased inflammation and cancer, whether in response to infection with a commensal organism or to chemical carcinogens[139]. A final mechanism is that certain immune responses can directly dampen cell-mediated anti-tumour immune surveillance mechanisms, thereby averting an immune reaction against the tumour that could potentially eliminate the cancer[90].

Handsfield and colleagues[35] cultured *N. gonorrhoeae* in expressed prostate fluid after urination in 93% of men with asymptomatic gonorrhea.

Viruses can also infect the prostate, and human papillomavirus (HPV), human herpes simplex virus type 2 (HSV2), cytomegalovirus (CMV) and human herpes virus type 8 (HHV8) have been detected in the prostate[36–38]. How frequently these agents infect the prostate, and whether they elicit an inflammatory response, is largely unknown. In conclusion, many different pathogens can infect the prostate. Whereas some of these are associated with inflammation, others have not been detected in association with inflammation. Because many additional bacterial sequences[39], and now a new viral sequence[40], can be found in prostate tissue in the absence of an ability to culture any of these organisms using traditional means, it is still possible that in analogy to *H. pylori* gastritis, researchers have missed a previously unidentified pathogen associated with most inflammatory lesions in the prostate.

Several epidemiological studies of STIs and prostate cancer have been undertaken (BOX 4). Adding weight to the argument for a link between inflammation and prostate cancer are data indicating that users of anti-inflammatory agents have a reduced risk of prostate cancer. Prospective and case–control studies, including a relatively small prospective analysis that we conducted, suggest a reduction of ~15–20% in the risk of prostate cancer in regular users of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) compared with non-users[41,42,43]; however, in a large study by Jacobs *et al.* the effect was seen only in long-term users[44].

Although most studies investigating clinical prostatitis in relation to prostate cancer reported a positive association[45,46], many of these studies might have been susceptible to detection bias. In our work, there was no association between clinical prostatitis and prostate cancer among men with an equal opportunity for prostate cancer screening by serum prostate specific antigen (PSA) testing, except in men diagnosed with cancer at a young age[47]. Although it is unclear why the effect was seen only in early-onset prostate cancer, it is possible that clinical prostatitis is associated with only a subset of prostate cancers that manifest at a relatively young age.

To determine whether inflammation is related to prostate cancer independent of clinical symptoms, it will be crucial to compare the patterns and extent of inflammation in prostate biopsy samples from men with and without carcinoma. As inflammation is so common in prostate specimens, these measurements will need to be quantitative, and will require large sample sizes. There is a US National Institutes of Health (NIH) consensus grading system[48] for histological prostate inflammation, and we will be using this system in a nested case–control study to determine whether asymptomatic prostatic inflammation is associated with prostate cancer using needle biopsy specimens from the Prostate Cancer Prevention Trial (PCPT)[49]. This trial is a large (approximately 18,000 men) study that was carried out to determine whether the 5 α-reductase inhibitor, finasteride, could reduce the period prevalence of prostate cancer. We will measure the pattern and extent of prostate inflammation and relate these to the presence or absence of prostate cancer.

Urine reflux, chemical and physical trauma. Chemical irritation from urine reflux has been proposed as an aetiological agent for the development of chronic inflammation in the prostate[50]. Although urine contains many chemical compounds that might be toxic

Prostatitis
Technically means 'inflammation of the prostate'. However, it is usually referred to as a clinical syndrome largely characterized by pelvic pain that has several subtypes. Some symptomatic subtypes (I and II) are associated with bacterial infections, others with inflammation but no infection (IIIa), or no inflammation and no infection (IIIb). Type IV consists of chronic inflammation without clinical symptoms.

Expressed prostate fluid
Secretions obtained following prostate massage after digital rectal examination.

Prostate specific antigen
A polypeptide that is expressed at very high levels in prostate epithelial cells, whereas very low levels are detected in normal serum; however, several pathological conditions such as prostate cancer, prostate inflammation and benign prostatic hyperplasia can result in increased serum PSA levels.

Table 1 | **Common somatic genetic and epigenetic changes in prostate cancer**

Gene and gene type	Location	Notes
Tumour-suppressor genes		
CDKN1B	12p13.1–p12	Encodes the cyclin-dependent kinase inhibitor p27. One allele is frequently deleted in primary tumours
NKX3.1	8p21.2	Encodes prostate-restricted homeobox protein that can suppress the growth of prostate epithelial cells. One allele is frequently deleted in primary tumours
PTEN	10q23.31	Encodes phosphatase and tensin homologue, which suppresses cell proliferation and increases apoptosis. One allele is frequently lost in primary tumours. Some mutations are found in primary tumours and more in metastatic lesions
TP53	17p13.1	Has many tumour-suppressor functions, including cell-cycle arrest in response to DNA damage, senescence in response to telomere dysfunction, and the induction of apoptosis. Mutations are uncommon early, but occur in about 50% of advanced or hormone-refractory prostate cancers
Oncogenes		
MYC	8q24	A transcription factor that regulates many target genes involved in cell proliferation, senescence, apoptosis and cell metabolism. Overexpression can directly transform cells. mRNA levels are commonly increased in all disease stages through unknown mechanism(s). Low-level amplification of the *MYC* locus is common in advanced disease
ERG	21q22.3	Proposed new oncogene for prostate cancer. Fusion transcripts with the 5′ portion of androgen-regulated gene (*TMPRSS22*) arise from deletion or chromosomal rearrangements commonly found in all disease stages
ETV1–4	7p21.3, 19q13.12, 1q21,-q23, 17q21.31	Encodes ETS-like transcription factors 1–4, which are proposed to be new oncogenes for prostate cancer. Fusion transcripts with the 5′ portion of androgen-regulated gene (*TMPRSS22*) arise from chromosomal rearrangements commonly found in all disease stages
AR	Xq11–12	Encodes the androgen receptor. Protein is expressed in most prostate cancers, and the locus is amplified or mutated in advanced disease and hormone-refractory cancers
Activation of the enzyme telomerase		Maintains telomere function and contributes to cell immortalization. Activated in most prostate cancers, mechanism of activation may be through MYC activation
Caretaker genes		
GSTP1	11q13	Encodes the enzyme that catalyses the conjugation of reduced glutathione to electrophilic substrates. Functions to detoxify carcinogens. It is inactivated in more than 90% of cancers by somatic hypermethylation of the CpG island within the upstream regulatory region
Telomere dysfunction	Chromosome termini	Contributes to chromosomal instability. Shortened telomeres are found in more than 90% of prostatic intraepithelial neoplasia (PIN) lesions and prostate cancer lesions
Centrosome abnormalities	N/A	Contributes to chromosomal instability. Centrosomes are structurally and numerically abnormal in most prostate carcinomas.
Other somatic changes		
PTGS2, APC, MDR1, EDNRB, RASSF1α, RARβ2	Various	The hypermethylation of CpG islands within upstream regulatory regions occurrs in most primary tumours and metastatic lesions. The functional significance of these changes is not yet known

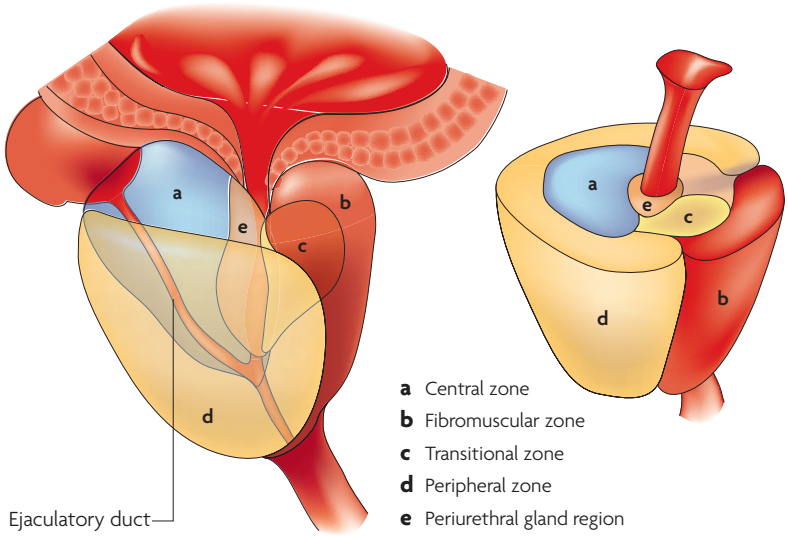
to prostate epithelium, uric acid itself might be particularly damaging[51]. In support of this, recent work has implicated crystalline uric acid as a 'danger signal' released from dying cells, and it has been shown to directly engage the caspase-1-activating NALP3 (cyropyrin) inflammasome present in cells of the innate immune system (primarily macrophages), resulting in the production of inflammatory cytokines that can increase the influx of other inflammatory cells[52]. In addition, urine reflux of injurious chemicals can function in conjunction with infectious agents to increase prostate inflammation. Another manner by which prostate inflammation might occur is the development of corpora amylacea[53] (FIG 2). Corpora amylacea have been proposed to contribute to prostate inflammation[54], persistent infection[55] and prostate carcinogenesis[56] because they are frequently observed adjacent to damaged epithelium and focal inflammatory infiltrates[54]. In terms of epidemiological research and corpora amylacea, few studies have been conducted, one of which observed a higher proportion of calculi in prostate tissue from patients with prostate cancer compared with patients with BPH[56], whereas others observed no association[57,58]. In support of the concept that 'flushing' of the prostate might be beneficial, several studies have found that increased ejaculation frequency, which might determine the rate of intraluminal corpora amylacea formation and the contact time between chemical agents such as uric acid or other urinary carcinogens and prostatic epithelium[59], is related to decreased prostate cancer incidence (REF. 60 and references therein). Spermatozoa have also been localized to prostate tissues, and the retrograde movement of sperm cells into the prostate has been found in association with inflammation[54,61] and with PIA lesions[61].

Inflammasome
A multiprotein intracytoplasmic complex that activates pro-inflammatory caspases, which then cleave the precursor of interleukin-1β (pro-IL1β) into the active form, leading to a potent inflammatory response.

Corpora amylacea
Amorphous small nodules or concretions located in the lumen of benign prostate acini and ducts that accumulate with age.

Dietary factors. Epidemiological studies have revealed a link between prostate cancer incidence and mortality and the consumption of red meat and animal fats[62–64]. One mechanism by which meats might stimulate cancer development could be related to the formation of heterocyclic amines (HCAs)[65]. The exposure of laboratory rats to dietary 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)[66] results in carcinomas of the intestine in both sexes, in the mammary gland in females and in the prostate in males[65]. Rodent prostates contain four different lobes that do not correspond anatomically to the zones of the human prostate, and PhIP induces cancer only in the ventral lobe of rats[67]. In a recent study we exposed laboratory rats to PhIP and found a similar increase in the mutation frequency in all lobes of the prostate, yet the ventral lobe selectively responded with increased cell proliferation and cell death[68]. Therefore, PhIP functions as both a lobe-specific classical 'tumour initiator' as well as a 'tumour promoter'. We also found that only the ventral lobe showed an increase in stromal mast cells, and stromal and intraepithelial macrophages[68]. After 12 weeks of PhIP exposure, the ventral lobe developed widespread epithelial atrophy; later, PIN and intraductal carcinomas were observed to develop directly from the atrophic epithelium (A.M.D., Y.N. and W.G.N., unpublished observations). Others have recently reported similar findings, in that PhIP treatment was found to induce inflammation and atrophy before inducing PIN and intraductal cancers[69]. Although it is not yet known whether the lobe-specific increase in mast cells and macrophages has a role in the neoplastic process, mast cells have been shown to stimulate cancer formation in several animal models, probably as a result of the release of factors such as tumour necrosis factor-α (TNFα) and various proteases, which might have an important role in tumorigenesis[70–72].

Prostate zones



	Prostate zone		
	Peripheral	Transition	Central
Focal atrophy			
Acute inflammation			
Chronic inflammation			
Benign prostatic hyperplasia			
High-grade PIN			
Carcinoma			

High prevalence
Medium-high prevalence
Low prevalence
None

Figure 1 | **Zonal predisposition to prostate disease.** Most cancer lesions occur in the peripheral zone of the gland, fewer occur in the transition zone and almost none arise in the central zone. Most benign prostate hyperplasia (BPH) lesions develop in the transition zone, which might enlarge considerably beyond what is shown. The inflammation found in the transition zone is associated with BPH nodules and atrophy, and the latter is often present in and around the BPH nodules. Acute inflammation can be prominent in both the peripheral and transition zones, but is quite variable. The inflammation in the peripheral zone occurs in association with atrophy in most cases. Although carcinoma might involve the central zone, small carcinoma lesions are virtually never found here in isolation, strongly suggesting that prostatic intraepithelial neoplasia (PIN) lesions do not readily progress to carcinoma in this zone. Both small and large carcinomas in the peripheral zone are often found in association with high-grade PIN, whereas carcinoma in the transition zone tends to be of lower grade and is more often associated with atypical adenomatous hyperplasia or adenosis, and less often associated with high-grade PIN. The various patterns of prostate atrophy, some of which frequently merge directly with PIN and at times with small carcinoma lesions, are also much more prevalent in the peripheral zone, with fewer occurring in the transition zone and very few occurring in the central zone. Upper drawings are adapted from an image on Understanding Prostate Cancer website. PIN, prostatic intraepithelial neoplasia.

Oestrogens. Another line of research into the causes of prostate inflammation and prostate cancer is the study of oestrogenic exposures in the prostate. Oestrogens are strongly linked to autoimmune processes in women, who are much more predisposed to autoimmune diseases than men. Increased levels of oestrogens, whether from environmental or developmental exposures, have long been linked to the development of prostate cancer[73,74]. Oestrogens affect the growth and development of the prostate, and this occurs through indirect routes on the hypothalamic–pituitary–gonadal axis through prolactin, and also by direct effects mediated by oestrogen receptor-α (ERα), which is expressed primarily in the stroma, and oestrogen receptor-β (ERβ), which is expressed primarily in the epithelium[73–76]. Oestrogens given to neonatal rodents result in an 'imprinted state' or 'developmental oestrogenization' in which there are developmental defects, including a reduction in prostatic growth. This treatment also results in the development of lobe-specific inflammation, hyperplasia and dysplasia or PIN[77,78]. Virtually all of these effects are mediated through ERα[79]. Therefore, it is quite plausible that chronic inflammation in the adult human prostate might reflect an autoimmune reaction caused, at least in part, by oestrogens.

A break of immune tolerance to prostate antigens? Another potential mechanism of self-perpetuating chronic inflammation in the prostate that could relate to all of the above-mentioned modes of prostate injury is that damaged prostate epithelial cells might release antigens that result in a break of the apparent immune 'tolerance' to the prostate. For example, many prostate

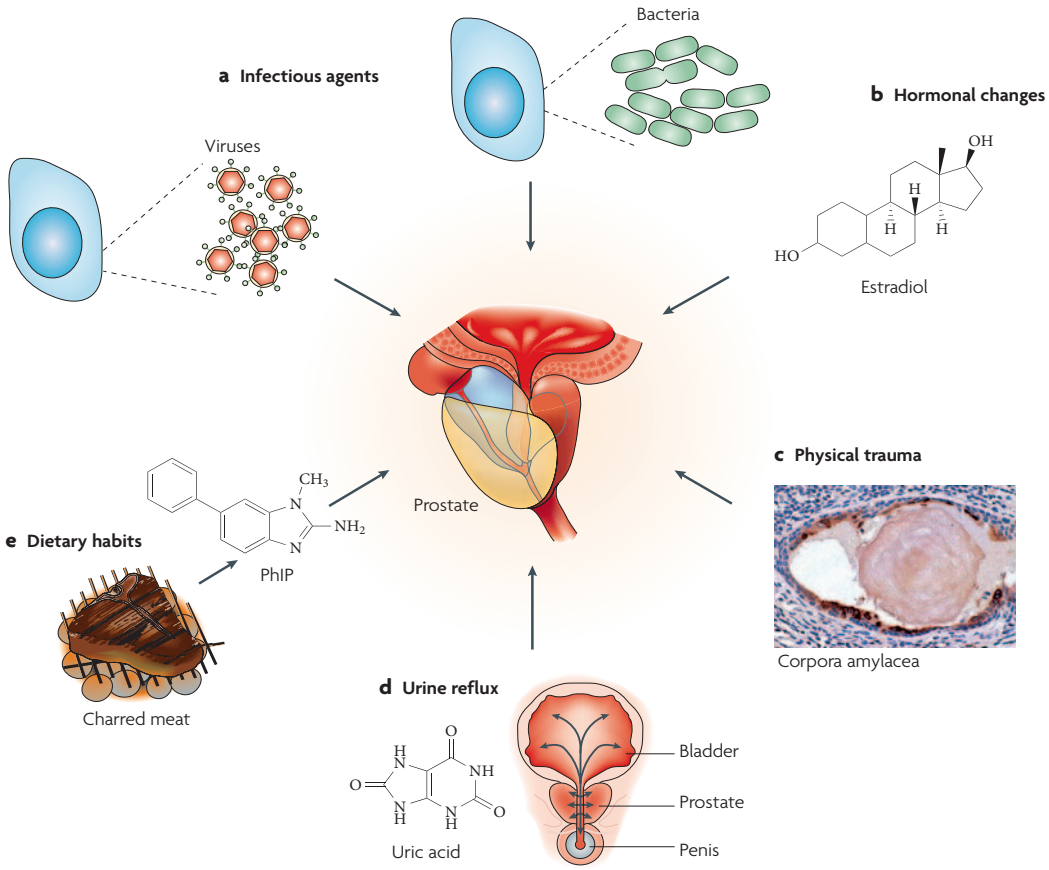


Figure 2 | **Possible causes of prostate inflammation. a** | Infection. Chronic bacterial prostatitis is a rare recurring infection in which pathogenic bacteria are cultured from prostatic fluid. Viruses, fungi, mycobacteria and parasites can also infect the prostate and incite inflammation. The figure represents two prostate cells infected either by bacteria or viruses. **b** | Hormones. Hormonal alterations such as oestrogen exposure at crucial developmental junctures can result in architectural alterations in the prostate that produce an inflammatory response. **c** | Physical trauma. Corpora amylacea can traumatize the prostate on a microscopic level. The figure shows a corpora within a prostatic acinus in which its edges appear to be eroding the epithelium, resulting in an increase in expression of the stress enzyme cyclooxygenase 2 (PTGS2), represented by brown immunostaining. Prostate cell nuclei are visible in violet following haematoxylin staining. **d** | Urine reflux. Urine that travels up back towards the bladder ('retrograde' movement) can penetrate the ducts and acini of the prostate. Some compounds, such as crystalline uric acid, can directly activate innate inflammatory cells. Although these compounds would not be expected to traverse the prostate epithelium, if the epithelium was already damaged this would facilitate the leakage of these compounds into the stromal space where they would readily activate inflammatory cells. **e** | Dietary habits. Ingested carcinogens (for example 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), which derives from charred meat) can reach the prostate through the bloodstream or by urine reflux and cause DNA damage and mutations, and result in an influx of inflammatory cells.

antigens are not expressed until after puberty, when the gland undergoes androgen-stimulated growth and development. This is likely to result in a lack of physiological immune tolerance to these antigens. Therefore, when released during prostate injury, these antigens could prime an immune response resulting in a specific reaction to prostate-restricted antigens. Indeed, a T-cell immune response to PSA in patients with chronic prostatitis has been reported[80].

In summary, many non-infectious mechanisms might lead to prostate epithelial cell and stromal damage. Injured cells are known to signal a 'danger response' that results in acute inflammation. Crystalline uric acid is particularly intriguing in this regard, as it directly interacts with a receptor that is part of a molecular pathway within innate immune cells that can potently stimulate inflammation. The fact that PhIP induces prostate inflammation and atrophy is also of great interest, as this might link diet to these processes in the prostate carcinogenesis pathway. Continuous exposure to the injurious agent can also set up the prostate for chronic inflammation that can lead to a sustained inflammatory response and cancer. Finally, all of these mechanisms of chronic epithelial injury might also result in a decreased barrier function, that could facilitate the growth of infectious agents that might further increase the inflammatory response, and allow toxic urinary metabolites into the prostatic interstitium, where they could further stimulate an inflammatory reaction. This is certainly an exciting area for continued research into the mechanisms of prostate carcinogenesis.

Heterocyclic amines
Molecules that are produced as a result of cooking meats at high temperatures, and which can be metabolized to biologically active, DNA-damaging agents. 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is the most abundant HCA.

Box 2 | **Somatic genomic alterations in PIA and proliferative atrophy**

In terms of somatic DNA alterations, although normal appearing epithelium (even from cancer patients) does not contain methylated glutathione *S*-transferase P1 (*GSTP1*) alleles, approximately 6% of focal atrophy lesions contain epithelial cells with methylated *GSTP1* (REF. 25). Another group found mutated p53 (REF. 140) and androgen receptor alleles[141] in post atrophic hyperplasia (a form of focal atrophy), prostatic intraepithelial neoplasia (PIN) and carcinoma, but not in normal prostate tissue — albeit these mutations in post atrophic hyperplasia were apparently non-clonal.

Others have used fluorescent *in situ* hybridization (FISH) to show that there are increases in chromosome 8 centromere signals[142,143], loss of chromosome 8p[143,144] and a gain of chromosome 8q24 in focal atrophy (REF. 143), indicating that chromosomal abnormalities similar to those found in PIN and carcinoma occur in a subset of these atrophic lesions. However, there were no atrophy cases in which clonal alterations were identified. Consistent with this, we recently found no evidence for clonal alterations indicative of chromosome 8 centromeric region gain, 8p loss or 8q24 gain in focal prostate atrophy, and infrequent 8p loss and absent 8q24 gain in PIN[27]. Therefore, the non-clonal mutations and non-clonal chromosomal alterations are probably indicative of genomic damage and/or the emergence of genomic instability in proliferative inflammatory atrophy (PIA) and proliferative atrophy.

In addition to the molecular evidence, other evidence to indicate that PIA and proliferative atrophy represent a field effect in the prostate is that these lesions are often quite extensive in the peripheral zone, often merge directly with high-grade PIN, at times merge directly with small carcinoma lesions and can be directly induced in the rodent prostate by exposures to the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP).

Immunobiology of prostate inflammation

The normal prostate, like all other organs, contains endogenous inflammatory cells consisting of scattered stromal and intraepithelial T and B lymphocytes[81,82], macrophages and mast cells. However, most adult prostate tissues contain increased inflammatory infiltrates, albeit the extent and type of inflammation are variable (for a review, see REF. 83). In terms of the biology of the inflammatory cells and the nature of the immune response in the prostate, most of the work has focused on BPH tissues in comparison with samples from the normal transition zone, and sometimes with carcinoma samples that have occurred in this region. Steiner *et al.* have examined the immunophenotypic and biological properties of chronic inflammatory cells in BPH and normal prostate tissues[84–86]. They have shown that of the increased CD45$^+$ cells (all leukocytes express CD45 and non-leukocytes do not), 70–80% of these are CD3$^+$ T lymphocytes, whereas 10–15% are CD19$^+$ or CD20$^+$ B lymphocytes. Macrophage numbers were also increased in these inflammatory lesions. In terms of the phenotype of the T cells, there is a reversed CD8:CD4 ratio, such that most T cells present in the normal areas expressed CD8, but most T cells in the inflamed areas expressed CD4. In terms of T-cell receptors (TCRs), 90% of the cells represent 'standard' αβ T cells (which express TCRαβ), with less than 1% representing γδ T cells. Class II major histocompatability antigen (HLADR), which indicates whether T cells are 'activated' by antigen signalling, is present on approximately 40% of the CD3$^+$ T cells, and many of these T cells expressed CD45RO, indicating that these are 'antigen experienced' T cells[85]. None of the T cells in the normal prostate epithelium showed evidence of either activation or of being antigen experienced T cells.

CD4$^+$ T cell responses can be divided into several different types that are classified according to their cytokine profile. T_H1 cells produce interferon-γ and TNFα, whereas T_H2 cells produce interleukin 4 (IL4), IL5 and IL13. Regulatory T (T_{Reg}) cells, which can suppress adaptive T-cell responses and autoimmunity, are characterized by the expression of CD25 and the transcription factor FOXP3, and they secrete transforming growth factor-β (TGFβ). In BPH, Marberger's group determined that the T-cell response is complex, in that although T_H0 (T cells that do not express any of the indicated cytokines) and T_H1 cells were predominant in the inflammatory lesions of BPH and in carcinoma, some features of a T_H2 response were also present. Unfortunately, at this point similar experiments have not been performed in the other zones of the prostate, or in areas of focal atrophy or PIN of the peripheral zone. The need for further understanding in this area is crucial, as is illustrated by the findings that microbially-driven inflammation can lead to colon cancer in mice, and that the prior transfer of T_{Reg} cells that express CD4 and CD25 prevents the inflammatory response that leads to colon cancer in these animals[87]. Recently Miller *et al.*[88], have shown that CD4$^+$ and CD25$^+$ T cells, with properties of T_{Reg} cells including the expression of the FOXP3 protein, are present in increased numbers in clinically localized prostate cancer tissues, compared with normal prostate tissues. Exciting new data from several groups suggest the importance of a new subset of CD4-effector T cells known as T_H17 cells, which develop through distinct cytokine signals (especially IL23) with respect to those involved in T_H1 and T_H2 responses, and are characterized by the production of IL17 (REF. 89). These cells are required for inflammation in arthritis and encephalitis models[89], and IL23 is required for skin cancer formation in response to carcinogen exposure in mice[90]. A potential role for T_H17 cells in prostatic inflammation had already been demonstrated by Steiner *et al.* before the T_H17 cell lineage had been recognized as being as distinct. They showed that activated T cells in BPH tissue and in prostate cancer express high levels of IL17 (REF. 85). Further work to more fully elucidate the phenotypic and biological properties of all T-cell subsets in the prostate is required before we can understand the significance of acquired cell-mediated immunity in prostate carcinogenesis. Methods such as the quantitative image analysis of immunohistochemically stained inflammatory cell subsets, as well as flow cytometry for these subsets using tissues isolated from histologically defined areas, will be crucial to obtain such data.

FOXP3
A nuclear transcription factor that is expressed specifically in regulatory T cells.

Box 3 | **Molecular pathways altered in PIA and proliferative atrophy**

In terms of molecular modes of action, p27 functions as an inhibitor of cell-cycle progression by inhibiting the activity of cyclin–cyclin dependent kinase complexes in the nucleus. Interestingly, p27 levels are generally reduced but not absent in human proliferative inflammatory atrophy (PIA), prostatic intraepithelial neoplasia (PIN) and prostate cancer[23,145–147]. The fact that p27 levels are not lost entirely (or biallelically inactivated by mutations) in cancer might be explained by recent findings that indicate that cytoplasmic p27 levels, which are increased by signalling through the MET receptor tyrosine kinase, are required for cell migration in response to hepatocyte growth factor signalling through MET and in response to increased cyclin D1 levels[148]. Therefore, although high levels of nuclear p27 can prevent cell-cycle progression, cytoplasmic p27 might be required for optimal tumour cell motility, which is a key feature of malignant transformation and tissue repair.

Phosphatase and tensin homologue (PTEN) is a dual protein and lipid phosphatase that is responsible for the dephosphorylation and inactivation of phosphatidylinositol 3,4,5-trisphosphate (PIP3), a second messenger produced after the activation of PIP3 kinase in response to the ligation of several growth factor receptors, including the insulin-like growth factor 1 receptor (IGF1R)[149]. PIP3 is required for the activation of the protein kinase AKT. AKT activation results in the inhibition of apoptosis and/or increased cell proliferation through several different effector mechanisms, such as the activation of mammalian target of rapamycin (mTOR) and S6 kinase.

NKX3.1 is a prostate-restricted homeodomain protein encoded within a region of chromosome 8p21 that often contains single copy deletions in prostate cancer[150]. In addition to suppressing the growth of prostate cells, decreased NKX3.1 protein levels result in increased oxidative DNA damage[151].

PIA and proliferative atrophy also show increased BCL2 protein expression[18], a gene product that is a potent suppressor of apoptosis. Other gene products that are increased in PIA and proliferative atrophy include those that are induced by oxidant and electrophilic stress, or by signals associated with cell activation and proliferation, including glutathione S-transferase P1 (GSTP1), GSTA1, cyclooxygenase 2 (PTGS2) and p16 (REFS 18,152,153). The fact that these stressed cells are undergoing tissue repair is supported by the finding that several proteins known to be involved in tissue repair and cell motility, such as MET[23] and HAI1 (REF. 154), have increased expression in PIA and proliferative atrophy.

Inflammatory genes and prostate cancer risk

Through a variety of approaches, including family and twin studies and segregation analyses, an important role for an inherited component of prostate cancer risk has been documented (recently reviewed by Schaid[91]). These studies have set the stage for efforts to identify prostate cancer susceptibility genes using linkage analysis and, more recently, association-based approaches. Despite strong evidence for a genetic component to prostate cancer risk, few reliable genetic risk factors for prostate cancer have been identified. In this section we will focus on a relatively new area of investigation in this field: the possibility that allelic variants of genes involved in innate and acquired immunity play an important part in determining inherited prostate cancer risk. If chronic inflammation is indeed an important aetiological factor for prostate cancer, then allelic variants of the genes involved in inflammatory pathways are logical candidates for genetic determinants of prostate cancer risk. As a result of space limitations, we can only review what we consider the most well-studied examples to date.

RNASEL and MSR1. Following up genomic regions of interest identified by linkage studies of prostate cancer families, two genes involved in innate immunity unexpectedly emerged as candidate prostate cancer susceptibility genes. Inactivating mutations (E265X and M1I) in ribonuclease L (*RNASEL*) segregate with prostate cancer in two prostate cancer families: E265X with one of European descent and M1I with a family of African descent[92,93]. *RNASEL*, which is located at 1q25, is a component of the innate immune system that is required for the antiviral and antiproliferative roles of interferons[94,95]. Lymphoblasts from carriers of either one of the mutations mentioned above were found to be deficient in enzymatic RNase activity, although, other than prostate cancer, additional phenotypic manifestations were not obvious. Subsequent studies examining the role of *RNASEL* as a prostate cancer susceptibility gene have provided mixed evidence, some confirmatory[91] and others not[91,96–99]. Although the association of this infection with prostate cancer development has yet to be shown[40], carriers of a common, hypomorphic allele of *RNASEL* (*R463Q*) were found to be at risk for prostatic infection by a new γ-retrovirus. Interestingly, when RNASEL is activated in cells by its cognate interferon-inducible ligands, 2′,5′-linked oligoadenylates, mRNA species are consistently induced, one of which is encoded by the (macrophage-inhibitory cytokine 1) *MIC1* gene, which is another prostate cancer susceptibility locus described below[100].

The analysis of candidate genes in a different region of linkage (8p22) in prostate cancer families revealed several recurring, inactivating mutations in macrophage scavenger receptor 1 (*MSR1*)[101]. The *MSR1* gene encodes a homotrimeric class A 'scavenger receptor', with expression largely restricted to macrophages. This receptor is capable of binding many ligands, including modified lipoproteins and both Gram-negative and Gram-positive bacteria. Mice with experimentally inactivated *Msr1* are more susceptible to various types of bacterial infection[102,103], although recent evidence suggests an anti-inflammatory role for this receptor, at least after exposure to certain pathogens[104,105]. *MSR1* mutant alleles, *R293X* and *H441R*, which are found in several different prostate cancer families, code for proteins that can no longer bind bacteria or modified LDL (C.M. Ewing and W.B.I., unpublished observations). Despite the initial findings of an increased frequency of *MSR1* mutations in men with prostate cancer, as with *RNASEL*, follow-up studies published on the possible role of *MSR1* variants and prostate cancer risk have yielded inconsistent results[98,101,106–111]. A recent meta analysis suggests that *MSR1* mutations might have a more reproducible effect on prostate cancer risk in African Americans[112]. Although these results do not indicate that these genes are major prostate cancer loci, they are consistent with these genes being able to modify prostate cancer risk, possibly in combination with particular environmental exposures — in this case, certain pathogens.

Toll-like receptors. The Cancer Prostate Sweden Study (CAPS) is a case–control study of prostate cancer in northern Sweden. The relative genetic homogeneity of the Swedish population and the large size of the CAPS study make it an ideal platform to identify genetic variants associated with prostate cancer risk. Studying cases and controls in CAPS over the past 3 years has led to the identification of several genes in inflammation-related pathways, including *MIC1*, interleukin 1 receptor antagonist (*IL1RN*) and members of the toll-like receptor (TLR) family, with allelic variants associated with prostate cancer risk.

As key players in innate immunity to pathogens, TLRs recognize pathogen-associated molecular patterns (PAMPs)[113]. The engagement of TLRs results in the production of various pro-inflammatory cytokines, chemokines and effector molecules, such as reactive oxygen and nitrogen intermediates, as well as upregulation of the expression of co-stimulatory CD86 and CD80 and major histocompatability complex II (MHC II) molecules, which facilitate adaptive immune responses. Ten members of the human TLR family have been identified, and for most of these, specific classes of ligands, typically microbial components or surrogates thereof, have been identified and characterized. Recently, sequence variants in several TLR genes have been linked to prostate cancer risk, including *TLR4* and the *TLR1–6–10* gene cluster[114,115].

Ligands that are recognized by TLR4 include Gram-negative bacterial products, including lipopolysaccharide[116], and human heat shock protein 60 (HSP60)[117]. In the CAPS study[114], a single nucleotide polymorphism (SNP) in the 3′ UTR region of *TLR4* (11381G/C) was found to be associated with prostate cancer risk. Carriers of the GC or CC genotypes of this SNP had a 26% increased risk of prostate cancer, and a 39% increased risk of early-onset prostate cancer (before the age of 65 years), compared with men with the wild-type GG genotype.

In a follow up study of a North American population, homozygosity for variant alleles of eight SNPs in *TLR4* (REF. 118) was associated with a statistically significantly lower risk of prostate cancer; however, the *TLR4*_15844 polymorphism, which corresponds to 11381G/C implicated in the CAPS population, was not found to be associated with prostate cancer. Therefore, although both published studies of this gene indicate that genetic variants of *TLR4* have a role in the development of prostate cancer, the specific variants responsible for this effect might vary across different populations.

The *TLR1–6–10* cluster maps to 4p14, and encodes proteins that have a high degree of homology in their overall amino-acid sequences[119]. TLR6 and TLR1 recognize diacylated lipoprotein and triacylated lipoprotein as ligands, respectively[120,121]. However, no specific ligand has been identified for TLR10. The TLR1 and TLR6 proteins each form heterodimers with TLR2 to establish a combinational repertoire that distinguishes a large number of PAMPs[120,122,123].

A study of the *TLR1–6–10* cluster in prostate cancer patients in CAPS identified an association of sequence variants in *TLR1–6–10* with prostate cancer risk[114,115]. The allele frequencies of 11 of the 17 SNPs examined in this gene cluster were significantly different between case and control subjects (P = 0.04–0.001), with odds ratios for variant allele carriers (homozygous or heterozygous) compared with wild-type allele carriers ranging from 1.20 (95% CI = 1.00–1.43) to 1.38 (95% CI = 1.12–1.70). Although further studies are necessary to understand the biological consequences of the risk variants in both *TLR4* and the *TLR1*–6–10 cluster, the observation of prostate cancer risk associated with polymorphisms in this family of genes, which is so intimately related to innate immunity, indicates that inflammation-related processes are important in prostate cancer development.

MIC1. MIC1 is a member of the transforming growth factor-β (TGFβ) superfamily, and is thought to have an important role in inflammation by regulating macrophage activity. In a study of 1,383 patients with prostate cancer and 780 control subjects in CAPS, a significant

Box 4 | **The epidemiology of STIs and prostate cancer**

Epidemiological studies of sexually transmited infections (STIs) and prostate cancer initially focused on gonorrhea and syphilis. Dennis and Dawson[155] combined the results of these studies and estimated summary odds ratios (ORs) of 1.4 for the development of prostate cancer in patients with a history of any STI, 2.3 for a history of syphilis and 1.4 for a history of gonorrhea. Similar estimates were also calculated in a subsequent meta-analysis[156]. Another recent case–control study reported that a history of both gonorrhea and more than 25 previous sexual partners were associated with an increased risk of prostate cancer[46]. The significance of many of these studies is limited, as most were small case–control designs that may have been susceptible to selection, recall and interviewer bias. In terms of other STIs, we recently observed that men who carried antibodies against *Trichomonas vaginalis* had a higher risk of prostate cancer than men who did not, and the association was stronger in men who rarely used aspirin[157].

In another inquiry we conducted a longitudinal study of young (median age <31) STI clinic patients by measuring serum prostate specific antigen (PSA) as a marker of prostate infection and damage. Men with an STI were more likely to have a ≥40% increase in serum PSA than men without an STI diagnosis (32% versus 2%, P <0.01)[158]. Increases in PSA levels were strongly suggestive of direct prostate involvement by the infectious agent, with resultant epithelial cell damage (either due to the organism itself or the inflammatory response to the organism) resulting in the release of PSA into the blood stream. As only about 32% of patients with acute STIs showed increased levels of PSA, it is apparent that either these agents do not always infect the prostate, or they do not illicit a strong inflammatory response that damages prostate tissues, or rapid antibiotic treatments prevent full-blown prostate involvement.

In terms of viral STIs and prostate cancer, Strickler and Goedert[36] concluded that those studied to date are unlikely to contribute to prostate carcinogenesis, although they did suggest the possibility of a causal relationship between an as-yet unresearched and unidentified infectious agent and prostate cancer. Urisman and colleagues[40] recently identified a novel γ-retrovirus in prostate tissues primarily from patients with germline *RNASEL* mutations. This intriguing finding is a proof of concept that specific infectious agents might persist in the prostate as a result of heritable changes in genes responsible for the clearance of these agents.

Odds ratio
The odds ratio is a way of comparing whether the probability of a certain event is the same for two groups, and is calculated using a 2×2 table. An odds ratio of one implies that an event is equally likely in both groups. An odds ratio greater than one implies that an event is more likely in the first group. An odds ratio less than one implies that the event is less likely in the first group.

Longitudinal study
A study in which repeated observations of a set of subjects are made over time with respect to one or more study variables.

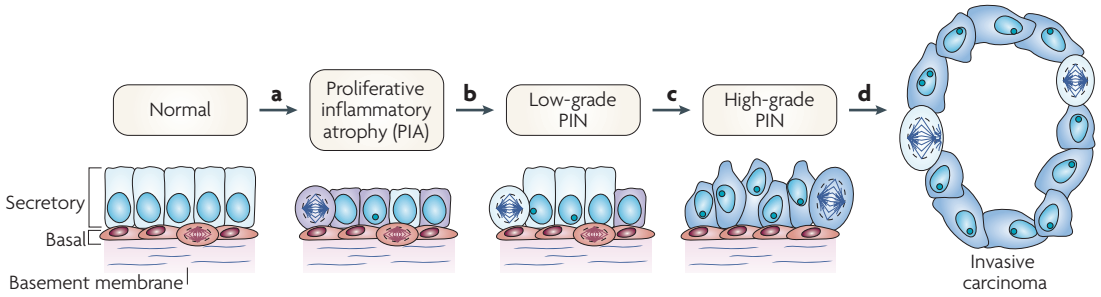


Figure 3 | **Cellular and molecular model of early prostate neoplasia progression. a** | This stage is characterized by the infiltration of lymphocytes, macrophages and neutrophils (caused either by repeated infections, dietary factors and/or by the onset of autoimmunity); phagocytes release reactive oxygen and nitrogen species causing DNA damage, cell injury and cell death, which trigger the onset of epithelial cell regeneration. The morphological manifestation of the cellular injury is focal prostate atrophy, which is proposed to signify the 'field effect' in the prostate. The downregulation of p27, NKX3.1 and phosphatase and tensin homologue (PTEN) proteins in luminal cells stimulates cell-cycle progression. Stress-response genes are induced (such as glutathione S-transferase P1 (*GSTP1*), *GSTA1* and cyclooxygenase 2 (*PTGS2*)). **b** | The subsequent silencing of *GSTP1* through promoter methylation in subsets of cells further facilitates oxidant-mediated telomere shortening. **c** | Cells carrying methylated *GSTP1* alleles and short telomeres have dysfunctional telomeres and are more likely to bypass the senescence checkpoints. This favours the onset of genetic instability and the consequent accumulation of genetic changes (for example loss of heterozygosity on 8p21,6q or gain of function on 8q24,17q). **d** | The continued proliferation of genetically unstable luminal cells and the further accumulation of genomic changes, such as gene rearrangements leading to *TMPRSS2–ETS* family member gene fusions, lead to progression towards invasive carcinomas. PIN, prostatic intraepithelial neoplasia.

difference ($P = 0.006$) in genotype frequency was observed for the non-synonymous change H6D between patients and controls[124]. Carriers of the GC genotype, which results in the H6D change, had a lower risk of sporadic prostate cancer (OR = 0.80, 95% CI = 0.66–0.97) and of familial prostate cancer (OR = 0.61, 95% CI = 0.42–0.89) than the CC genotype carriers. In the study population, the proportion of prostate cancer cases attributable to the CC genotype was 7.2% for sporadic cancer and 19.2% for familial cancer.

IL1RN. The protein product of the *IL1RN* gene belongs to the interleukin 1 cytokine family of proteins. Its primary function is as an inhibitor of the proinflammatory IL1α and IL1β. Lindmark *et al.* examined four haplotype-tagging SNPs (htSNPs) across the *IL1RN* gene in samples from patients with prostate cancer[125]. The most common haplotype (ATGC) was observed at a significantly higher frequency in the cases (38.7%) compared with the controls (33.5%) ($P = 0.009$). Carriers of the homozygous ATCG haplotype had significantly increased risk (OR = 1.6, 95% CI = 1.2–2.2). Furthermore, the association of this haplotype was even stronger among patients with advanced disease compared with controls[125].

Other inflammatory-related genes

Many other genes in inflammatory pathways have been examined recently for a link to prostate cancer, generally with mixed results. For example, although McCarron *et al.* previously reported an association between certain alleles of *IL10* and *IL8* and prostate cancer[126], Michaud *et al.* recently reported a lack of association of polymorphisms in the *IL1β*, *IL6*, *IL8* and *IL10* and prostate cancer in a case–control study of the Prostate, Lung, Colorectal, and Ovarian Cancer screening trial[127]. Further work is necessary to either confirm or refute the hypothesis that variants in genes associated with inflammation affect prostate cancer risk, and if confirmed, to understand the mechanisms that link allelic variation in inflammation genes and prostate cancer.

SNPs and the inflammatory pathway

In a more global genome-wide approach, Zheng *et al.*[128] proposed that sequence variants in many other genes in the inflammatory pathway might be associated with prostate cancer. They evaluated 9,275 SNPs in 1,086 genes of the inflammation pathway among 200 familial cases and 200 unaffected controls selected from the CAPS study population. They found that more than the expected numbers of SNPs were significant at a nominal P value of 0.01, 0.05 and 0.1, providing overall support for the hypothesis. A small subset of significant SNPs (N = 26) were selected and genotyped in an independent sample of ~1,900 members of the CAPS population. Among the 26 SNPs, six were significantly associated with prostate cancer risk ($P \leq 0.05$). These results are consistent with the idea that variation in many genes in inflammatory pathways might affect the likelihood of developing prostate cancer.

Ideally, one would prefer to correlate the presence of specific genetic polymorphisms with the pattern and extent of intraprostatic inflammation, yet in all of the studies reported above the status of the prostate in men in terms of presence, pattern and extent of inflammation is unknown. Future studies that address these issues will be crucial in evaluating the biological effects of various polymorphisms in inflammatory pathway genes.

The 'injury and regeneration' hypothesis

Our current working model (FIG. 3) suggests that repeated bouts of injury (and cell death) to the prostate epithelium occur, either as a result of oxidant and/or nitrosative damage from inflammatory cells in response to pathogens or autoimmune disease, from direct injury from circulating carcinogens and/or toxins derived from the diet or from urine that has refluxed into the prostate. The morphological manifestation of this injury is focal atrophy or PIA, which we postulate to be a signature of the 'field effect' of prostate carcinogenesis. The biological manifestations are an increase in proliferation and a massive increase in epithelial cells that possess a phenotype intermediate between basal cells and mature luminal cells[5,6,23]. In a small subset of cells, perhaps cells with an intermediate phenotype that contain at least some 'stem cell' properties, somatic genome alterations occur, such as cytosine methylation within the CpG island of the *GSTP1* gene and telomere shortening. Both of these molecular changes can decrease the 'caretaker' phenotype and increase genetic instability that might then initiate high-grade PIN and early prostate cancer formation. In the setting of ongoing inflammatory and dietary insults in cells with compromised caretaker functions, additional changes such as gene rearrangements resulting in the activation of the ETS family of oncogenic transcription factors, the activation of *MYC* expression and the loss of tumour-suppressor genes such as *PTEN*, *NKX3.1* and *CDKN1B* occur that drive tumour progression.

Future directions

We reviewed evidence that in men with an underlying genetic predisposition, prostate cancer might be caused by inflammation possibly coupled with dietary factors. However, additional work needs to be done to determine whether the mechanisms proposed are correct. First, we need an improved ability to diagnose and define clinical 'prostatitis'. Second, we need studies that quantify asymptomatic inflammation in the prostate to determine the relationship between the development of prostatic inflammation and the following: age, genotype, response to specific infectious organisms, and diet. We need an improved understanding of the types of inflammatory cells and their biological properties in the normal prostate and in the various lesions such as PIA, BPH, PIN and carcinoma. Another potential avenue for future studies is to couple improvements in imaging of the prostate, including new strategies to image inflammation and atrophy, to studies aimed at quantifying various types of inflammation in prostate biopsy specimens and quantitative analyses of cytokine profiles and inflammatory cell types in prostate fluid. These studies should also be performed in conjunction with experiments designed to identify specific infectious organisms. It will be crucial in these studies to have both the genetic information and the dietary and medical history data to correlate with the immunobiological data. Improvements in our understanding of the key molecular genetic and epigenetic events that drive prostate carcinogenesis, and the identification of the precise cell types involved (that is, whether prostate epithelial stem cells or their progeny are directly transformed) need to be applied to presumed precursor lesions to define precisely the order of events in the development of early prostate cancer. As animal models of prostate cancer continue to be developed that mimic the human disease, such as those that activate *MYC* or inactivate *PTEN*, *CDKN1B* or *NKX3.1* (REFS 28,129), strategies for determining whether infectious agents and/or specific activated inflammatory cells are required for prostate carcinogenesis also need to be developed. Examples of such studies include crossing mice that are genetically engineered to develop prostate cancer with mice that lack specific subsets of cells of the innate and adaptive immune systems, to determine the contribution of such cells to the transformation process. In other studies, one can target inflammation to the prostate by using transgenic technologies to overexpress chemokines and/or cytokines that attract inflammatory cells to the prostate or that will activate inflammatory cells that are already resident in the prostate. As rodents are quite resistant to prostate cancer development, these studies would be potentially more informative if they were carried out on genetically altered animals already prone to developing early neoplastic lesions in the prostate.

Inflammation is a very complex process, which involves hundreds of genes. Therefore, there are many genes in the inflammatory pathways that might contribute to the development of prostate cancer. Whereas many genes in the inflammatory pathway have been shown to harbour sequence variants that may or may not be associated with increased risk of prostate cancer, larger studies in different study populations are needed to confirm and more thoroughly characterize the associations discussed above. Traditional association tests that examine one gene at a time remain valuable approaches, but they are fast being supplanted by new approaches that provide an efficient and economically feasible way to study virtually all of the genes in the whole pathway. Technologies using bead-based or chip-based arrays allow for the rapid examination of thousands of SNPs among hundreds of genes, and even genome-wide searches assessing all genes. Such approaches will provide a comprehensive evaluation of genes in inflammatory pathways, and will provide an appropriate perspective of the importance of genes in these pathways in the context of all known cellular pathways. Although the data obtained so far using genome-wide scans are promising, the challenges of such studies are significant, as many associations are expected to occur by chance. Only when specific associations are validated in multiple large cohorts will the scientific community have confidence in the purported findings from such studies. Although it is currently unknown whether or not 8q24 is related to inflammatory pathways, one very recent example of a success story stems from a set of independent studies that implicated this chromosome region in prostate cancer occurring in both families and in sporadic cases[130,131].

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Competing interests statement
The authors declare no competing financial interests.

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The following terms in this article are linked online to:
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- Abstract:

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Paradoxical downregulation of C-MYC and upregulation of NKX3.1 proteins during invasion in the Lo-MYC mouse prostate

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Abstract:
Introduction and Objective: *C-MYC* has been implicated in human prostate cancer and targeted overexpression of *C-MYC* in the mouse results in prostatic intraepithelial neoplasia (PIN) and invasive adenocarcinoma (CaP) (Cancer Cell. 4:223-238, 2003). However, the onset and dynamics of C-MYC protein expression, and its relation to morphological transformation, the expression of other prostate tumor suppressor proteins, early invasion and the development of inflammation have not been addressed. *Methods:* Lo-MYC and wild type mice were sacrificed at various ages up to 52 weeks. The prostate lobes were dissected and processed for histological and immunohistochemical analysis for C-MYC, androgen receptor (AR), Nkx 3.1, p27, Ki67, cleaved caspase 3, F4/80, CD3, CD45, p63 and smooth muscle actin. *Results:* All mice showed PIN at 4 weeks of age with no invasion until 52 weeks. The morphological alterations in PIN included an increase in cell and nuclear size, nucleolar enlargement, hyperchromasia, increased mitoses and apoptotic bodies. Detection of C-MYC protein coincided with morphological alterations, suggesting that C-MYC is sufficient for transformation. All mice showed microinvasive CaP by 52 weeks, and both the level of C-MYC protein and the degree of cytological atypia were decreased upon early invasion, with recovery of C-MYC protein and nuclear atypia upon enlargement of the invasive tumor. Gland formation and AR expression were maintained during early invasion. The expression of Nkx 3.1 was inverse to that of C-MYC. Decreased p27 and increased Ki67 and cleaved caspase 3 were found in PIN and CaP. Inflammatory cells, consisting of mostly of CD45 positive lymphocytes and F4/80 positive macrophages increased in an around the lesions as they progressed. *Conclusions:* We verified that Lo-MYC mice develop PIN and early CaP that resemble the human disease. Our new data show: (i) C-MYC is sufficient to morphologically transform prostate cells into PIN; (ii) C-MYC protein is decreased and Nkx3.1 protein is increased transiently during invasion; (iii) inflammatory cell infiltrates accompany the development and progression of PIN to CaP. These results validate the ability of the Lo-MYC mouse to model early human CaP and reveal dynamics of C-MYC and Nkx3.1 protein expression and inflammation during disease progression.
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